PATENT
Attorney Docket No.: 021911-000600US
Client Ref. No.: OBM15

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Lewis, *et al.*

Application No.: 09/284,009

Filed: April 5, 1999

For: MONONUCLEAR PHAGOCYTES IN THERAPEUTIC DRUG DELIVERY

Examiner: C. Qian

Art Unit: 1636

DECLARATION UNDER 37 C.F.R. §1.132 BY STUART NAYLOR, Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Stuart Naylor, Ph.D., am Vice President: Biological Systems at Oxford BioMedica, the assignee of the above identified application. I have over 17 years of experience in molecular and cellular biology and immunology and have been an author of numerous publications in peer reviewed scientific journals in this field. My current responsibilities include Oxford BioMedica's macrophage-based gene delivery programme.

2. My *curriculum vitae* was previously provided with my Declaration of April 24, 2002 as filed in the above identified application, which Declaration is hereby incorporated by reference. Briefly, I received my Bachelor of Science degree in Microbiology and Virology in 1984 from University of Warwick and my Ph.D. in

Tumour Biology in 1992 following completion of studies at the Imperial Cancer Research Fund. After obtaining my doctorate, I spent a further 5 years in postgraduate study at two of the top Cancer Institutions in Europe: the Imperial Cancer Research Fund and the Institute of Cancer Research (ICF), specializing in the role of cytokines in epithelial cancer biology with particular reference to macrophage derived cytokines such as tumour necrosis factor. More specifically at ICF, I spent four years focused on the role of macrophages in the tumour microenvironment with reference to migration, tumouricidal activity and matrix remodeling (see references attached to my Declaration of April 24, 2002).

3. I have read and am familiar with the contents of the above identified patent application and claimed subject matter. It is my understanding that the Examiner has rejected the claims as allegedly unpatentable to the extent that the claims encompass mononuclear phagocytes comprising one regulatable element operably linked to at least one nucleotide sequence of interest (NOI) wherein the scope of nucleotide sequence exceeds beyond "a marker or reporter gene".

4. This declaration is provided to show that following the description and guidance provided by the above identified application, an NOI encoding a therapeutic gene product was operably linked to a regulatable element as described in the application and expressed in mononuclear phagocytes to treat tumor cells *in vivo*.

5. This declaration is also provided to show that the description and guidance provided by the above identified application may be used to prepare phagocytes to treat other conditions.

6. As part of my responsibilities with Oxford BioMedica's macrophage-based gene delivery programme, I have reviewed, and am familiar with the content described in paragraphs 7, 10-12 and 14-15, as well as the associated figures and table, below. The described work was conducted under my direction.

7. MetXia-MG refers to macrophages that have been transduced with an adenovirus bearing the cytochrome P450 gene isoform 2B6 (hereafter P4502B6) under the control of, and thus operably linked to, a hypoxic response element (HRE) promoter and the P450 reductase gene under the control of the CMV promoter. This adenovirus vector is designated AP48c.

8. The construction and arrangement of AP48c used routine methods known in the art as described on page 14, line 5, to page 15, line 11 of the above identified application, for example. The methods and results are analogous to those described on pages 21-27, Examples 1 and 2 as well as Figure 4, of the application. The application also recites the possible expression of P4502B6 as an NOI on page 8, lines 23-24.

9. The methods and results are also analogous to those described by me and my colleagues in Griffiths et al. (Gene Therapy, 7:255-262, 2000), a copy of which is attached hereto.

10. The model HU is an ascitic ovarian cancer xenograft model. Upon treatment with intra-peritoneal (i.p.) TNFα (200ng/mouse for seven days), HU converts from the ascitic form into a solid tumour.

11. Figure 1 shows a diagrammatic overview of the methodology used. On day 0, 60 nude female mice were injected with 0.2 ml HU-ascites i.p. On day 7 mice received TNFα i.p. daily for seven days. Mice were split into groups of 10 mice each and were administered i.p. with 0.2ml buffer, untransduced macrophages (2×10^6) or 2×10^6 macrophages transduced with AP48c according to Table 1.

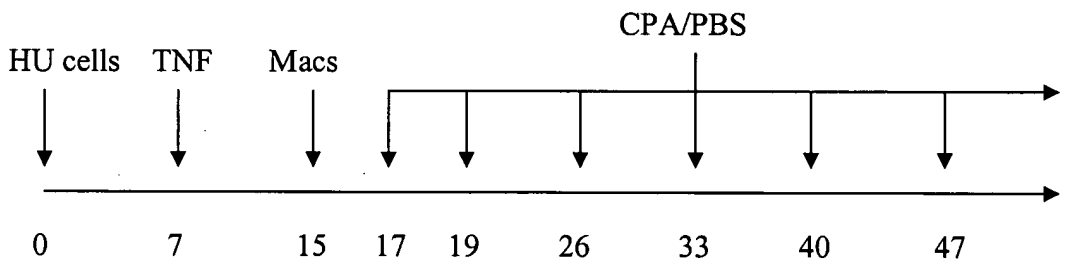


Figure 1. Overview of study design.

TABLE 1. OVERVIEW OF STUDY GROUPS

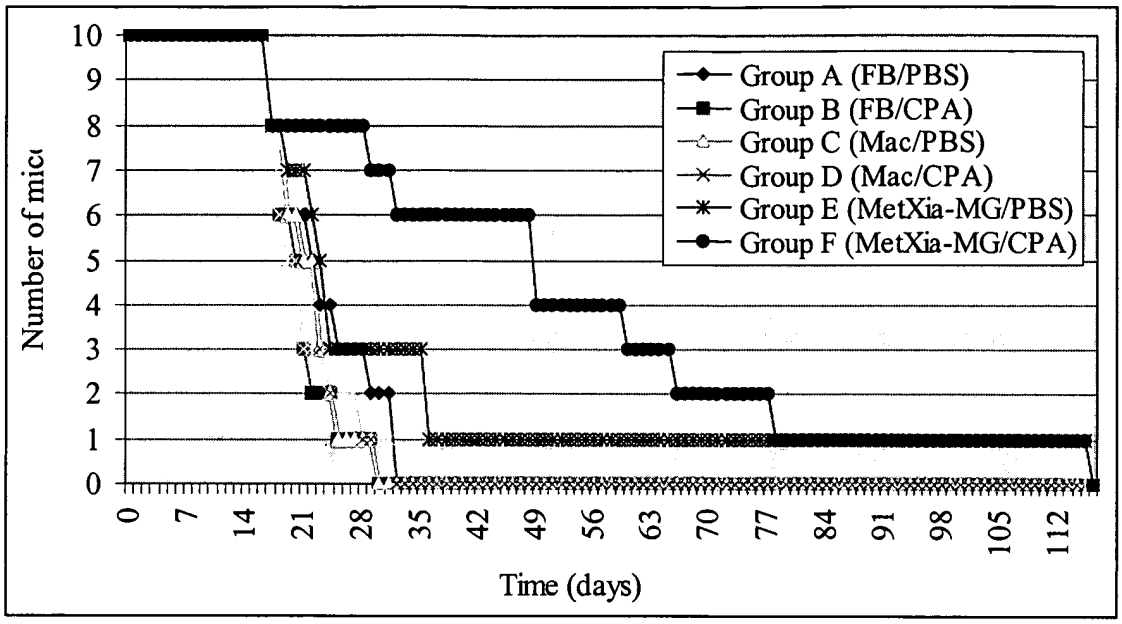
Group	Test Article 1	Test Article 2
A	Formulation buffer	PBS
B	Formulation buffer	CPA
C	Untransduced macrophages	PBS
D	Untransduced macrophages	CPA
E	MetXia-MG (macrophages transduced with AP48c)	PBS
F	MetXia-MG (macrophages transduced with AP48c)	CPA

12. Two days later two mice per group were euthanised and tissues (liver, kidneys, lungs, heart, spleen, lymph nodes) collected for gene transfer analysis. The remaining mice were administered 0.2 ml PBS or cyclophosphamide (CPA) at 1 mg/mouse according to Table 1 on day 17, 19 and then every seven days.

13. The prodrug cyclophosphamide is activated by the P4502B6 gene product to the active metabolite 4-hydroxy-cyclophosphamide which kills tumour cells (see Griffiths et al., attached hereto). The active metabolite thus exhibits a "bystander" effect wherein non-transduced cells in the vicinity of transduced cells producing the active metabolite are also killed effectively. Another gene product capable of producing the "bystander" effect is Herpes simplex virus thymidine kinase (HSV-tk) gene expression with ganciclovir (GCV) treatment, which is discussed for use in the above identified application on page 8, lines 14-21. Successful *in vivo* expression of HSV-tk (using a retroviral vector like that described in the above identified application on page14, lines 5-15) in the presence of GCV to kill cells is described by Nishihara et al. (Endocrinol. 138(11):4577-4583, 1997), a copy of which was submitted in the above identified application in 2003 and initialled by Examiner Qian on November 10, 2003 (see page 4580, right column, last paragraph, to page 4581).

14. The mice in the various groups were observed for signs of toxicity and followed to survival. Post-mortem analysis was performed and tissues collected for histological analysis. Figure 2 illustrates the survival advantage of tumour-bearing mice receiving MetXia-MG in combination with cyclophosphamide.

Figure 2



15. As shown by the results of this study, tumour-bearing mice treated with a single administration of MetXia-MG in combination with repeated administrations of CPA were provided with a survival advantage in comparison to control mice. This survival advantage exceeded that of mice treated with either CPA alone or in combination with untransduced macrophages.

16. As one of skill in the art, I declare that the above results indicate that the adenoviral vector AP48c was able to express sufficient amounts of P4502B6 to activate CPA and produce sufficient tumour cell killing to result in a therapeutic effect *in vivo*. This resulted in enhanced survival of the mice over time.

17. I further declare that the expression of sufficient amounts of P4502B6 was a result of transcription regulated by the operable linkage of the P4502B6 encoding sequence to the HRE promoter, a regulatable element as described in the application and encompassed by the pending claims therein. The HRE promoter is known to direct expression under hypoxic conditions, such as those within solid tumors (see Griffiths et al., attached hereto).

18. I also declare that there is no objective reason to limit the above results to mice or the ovarian tumor model used. The fundamental basis of tumour infiltrating phagocytes, such as macrophages, that express an NOI under the hypoxic conditions

found in a tumour to kill tumour cells, is equally valid in non-murine animals, including humans, and with other tumour types.

19. The ability to use the above approach with other tumours as well as other disease is reflected in Griffiths et al. (attached hereto) on page 260, right column, second paragraph, through page 261, left column, first full paragraph. Essentially, the above approach, which is but one embodiment of the approach described in the above identified application, provides a valid therapeutic strategy for the treatment of cancer by using modified phagocytes that target tumours. This includes breast tumours (reflected by the T47D breast cancer cell line treated in Griffiths et al., attached hereto), prostate tumours, bladder tumours, liver tumours, pleural tumours, metastatic and disseminated tumours, or tumours of other tissues, including the head and neck. The above approach may also be applied to other conditions characterized by hypoxia, such as arthritis (inflammatory disease) and ischaemic (cardiac) disease. Such a statement published in a respected and respected journal could only have been made if the reviewers of the manuscript were in agreement with the views being set forth.

20. The statements by Griffiths et al. are also consistent with subsequent work described by Kluth et al. (J. Immunol. 166:4728-4736, 2001), a copy of which was submitted in the above identified application in 2003 and initialled by Examiner Qian on November 10, 2003. Kluth et al. describe the use of macrophages transfected with adenovirus vectors expressing interleukin-4 (IL-4) to treat an inflammatory condition *in vivo*. The macrophages were able to localize to the sites of inflamed glomeruli (see page 4730, right column, bottom, to page 4731, as well as Figures 2 and 3).

21. I agree with the statement by Kluth et al. (see page 4728, abstract, last sentence) that "[m]acrophage transfection and delivery provides a valuable system to [] modulate inflammatory disease and highlight the feasibility of macrophage-based gene therapy." I find no objective reason to limit doubt that the results observed and described by Kluth et al. would also occur with the use of analogous vectors to express an NOI as described in the above identified application. The fundamental basis of using phagocytes, such as macrophages, that localize to sites of inflammatory disease, is equally valid when expressing gene products other than IL-4 as well as when in non-rat animals, including humans.

22. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: _____ By: _____
Stuart Naylor, Ph.D.

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